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Identification of Cyanobacterial Communities and Their Microcystin Congeners in Soda Pits at Nyala Magnesite Mine, South Africa

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Abstract Cyanobacterial species are known to inhabit adverse environments ranging such as brackish to freshwater. Here we report on the identity of cyanobacterial species and their microcystin congeners that were found in soda pits of an abandoned Nyala Magnesite mine, Limpopo province, South Africa. The samples were collected from soda pits 1 and 3 (water) and pit 3 (sediments) which was later cultured in BG 11 liquid media, incubated at room temperature under continuous light. The fluid samples were introduced to FlowCAM instrument to obtain images for subsequent identification. The algal species that were found in the soda pits were: Microcystis, Oscillatoria, Anabaena, Cylindrospermopsis and Phormidium. The microcystin congeners were identified by High Performance Liquid Chromatograph-Diode Array Detector (HPLC-DAD). The HPLC-DAD analysis showed the presence microcystin congeners LR, YR, LY and their range levels of 24.35 to 102.71, 18.16 and 28.93 ppb respectfully. The microcystin RR was absent. The microcystin LR level exceeds the WHO guidelines of 1 ppb. The availability water in the soda pits has attracted the local communities to use the soda pits for fishing, stock watering and recreational swimming. Thus the presence of these microcystins congeners has the potential to cause harm to the local communities.
Keywords cyanobacterial communities, microcystin congeners, abandoned alkaline mine tailings, Microcystis

Introduction

Open cast mining creates pits which are flooded with water over time and upon closure and can be hazardous to the environment. Nyala Magnesite mine has been inactive for many years and the pits are now filled with brackish and alkaline waters (Jeleni et al. 2012). Carmichael (1994) stated that algae do strive in fresh or brackish waters if the nutrients and solar irradiance levels are favourable. The growth of these algae in eutrophic lakes can be an aesthetic nuisance and can cause odour problems (Keneck et al. 1992). Freshwater and brackish water toxic blooms has been reported in many places like Brazil, Canada, Europe, India, Japan, China, South Africa, USA (Carmichael, 1989). Heavy blooms of algae in water kill animals such as livestock, wild animals, and waterfowl (Codd et al. 2005; Oberholster et al. 2009)

According to Codd et al. (1989) blue-green algae, Anabaena, Aphanizomenon, Microcystis, Nodularia and Oscillatoria are the algal species that were found to be dominant in South African fresh and brackish waters. Microcystis is the most dominate cyanobacteria and is linked to the production of microcystins, of which there are 80 congeners (Van Vuuren et al. 2006). Carmichael (1989) reported that algae is always present in the water, but only become of concern when harmful algal blooms occur that often lead to fish mortalities. The soda pits in Nyala Magnesite mine have stagnant water thus making them prone to having algal blooms due to nutrients enrichments of domestic animals like cows and donkeys which drink the water. The main objectives were to identify the algae and their microcystin congeners in the soda pits. This is important since the waters in the soda pits are used stock watering and for recreational swimming.

Materials and methods

Location of the study area

The abandoned Nyala Magnesite Mine tailings are located at Folovhodwe village in Zwigodini area which falls under Mutale local Municipality in Limpopo province, South Africa. The mine lies between 22°31′52.48″South and 30°37′15.02″ East of Latitude and longitude respectively, and approximately 475 m above sea level. The coordinates that were recorded in soda pits 1, 2, 3 are the following respectively: 22°31′52.48″ S 30°37′15.02″ E, 22° 31′40″S 30°37′50″E and 22°34′44″S.

Sample collection

The two water samples were collected by simply filling up the 250 ml sterile water bottles. The third sample was collected by using a sterile swab which was rubbed against the dried soda pit soil and then transferred to 250 sterile glass bottle which contained BG11 liquid media. The BG11 liquid media was prepared as per procedure of Gumbo and Cloete (2011). The water samples were subdivided for identification of cyanobacteria species and microcystin analysis.

Sample analysis

The culturing of algae

The three water bottles were incubated at room temperature under a continuous light (1076±204 lux) so allow the cyanobacteria to grow for 30 days. The cyanobacteria that was growing in the sample bottles was then identified by the use of a FlowCAM.

Algae identification

A bench top FlowCAM was used to capture images which were then used to identify the cyanobacteria image by comparison to literature.

Microcystin analysis

The water samples were sent to University of Johannesburg for microcystin analysis.

Results and discussion

The identification of cyanobacteria communities in the soda pits

The filamentous and colonial cyanobacteria were found in the soda pits (table 1; fig 1). The cyanobacteria species were tentatively identified by using pictures captured by the Flowcam and then compare with published pictures available in the literature. The filamentous cyanobacteria species seemed to be the dominate species in all the soda pits, the least dominant species was *Phormidium* spp which was identified only once in soda pit 3. The highest number was in soda pit 3, where 5 Cyanophyta were identified. This may be attributed to deliberate provision of nutrients in the BG 11 media which allowed the cyanobacteria to strive. The lowest number of species was found in soda pit 1 and 2, where only 3 Cyanophyta are identified.

Table 1 *Cyanobacteria present in the sampled soda pits*

Species	Soda pit	References
Oscillatoria	1,2,3	van Vuuren et al. 2006
Microcystis	1,3	van Vuuren et al. 2006
Phormidium	3	Lopez 1998
Anabaena	2,3	van Vuuren et al. 2006
Cylindrospermopsis	1,2,3	van Vuuren et al. 2006

Kamennaya et al. (2012) and Renaut and Stead (1990) have reported the presence of cyanobacterial blooms occurring in Magnesite dominated sites. There is no doubt that harmful algal blooms are occurring in more locations than ever before and new sightings are reported regularly (Sellner et al., 2003). A study done by Hamed et al. (2007) in the Saline-Alkaline Lakes of Wadi El- Natrun Egypt it shows that a total of 86 cyanobacterial taxa were identified and from the species Identified were Microcystis spp, Gloeocapsa spp, Synechocystis spp, Isocystis spp, Gomphosphaer spp, Merismopedia spp, Dactylococcopsis spp, Labyrinthiformis spp, Spirulina spp, Oscillatoria spp, Anabaenopsis spp, Anabaena spp, Pseudanabaena spp, etc. A study by Jonker et al. (2013) in thermal springs in Limpopo province found Cyanophyta, Bacillariophyta, Chlorophyta, Euglenophyta and Dinophyta. From these taxa Oocystis, Coelastrum, Anabaena, Cocconeis, Peridinium. Scenedesmus and Chlamydomonas, Oscillatoria Synedra, Aulacoseira, Nitzschia, Cyclotella, Gyrosigma, Craticula Cymbella, Pinnularia and Navicula. The species that are common to those of Nyala Magnesite soda pits are Anabaena, Oscillatoria and Microcystis.

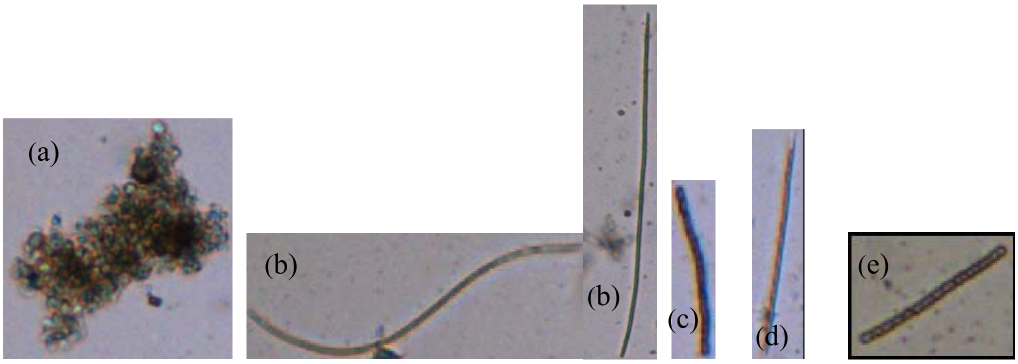

Fig. 1 *Cyanobacteria species in the soda pits (a) Microcystis; (b) Oscillatoria; (c) Anabaena and (d) Cylindrospermopsis* (van Vuuren et al. 2006); (e) *Phormidium* (Lopez 1998)

The production of microcystins by algae

The results showed the three variants of microcystins (table 2). For microcystin RR it was not found in any of the three soda pits. In three soda pits the level of microcystin LR was the highest (102.71 ppb) followed by microcystin LY (28.93 ppb) and microcystin YR (18.16 ppb).

Table 2 *Microcystin congeners present in the soda pits*

Microcystin variants	Soda pit 1	Soda pit 2	Soda pit 3
Microcystin RR (ppb)	---	---	---
Microcystin LR (ppb)	32.12	102.71	24.25
Microcystin YR (ppb)	---	18.16	---
Microcystin LY (ppb)	---	28.93	---

Not detected in the water samples

Our results show that the levels of microcystin LR (table 2) exceed the WHO provisional guideline value of 1 ppb microcystin LR for drinking water and 20 ppb for recreational water (WHO, 1998). The presence of microcystins in the soda pits may pose a health hazard to the communities around the soda pits since the water is used for a variety of uses (Figure 2). Microcystin toxicity has been implicated in death of 50 dialysis patients in Caruaru Brazil in 1996 (Pouria, 1998). Epidemiological studies have shown that long term exposure to microcystins via drinking water supplies has been associated with primary liver cancer (Falconer et al. 1983).

Fig. 2 *showing livestock and people collecting water and washing clothes around the soda pit 2*

Conclusion

The cyanobacterial species that were recorded from the Nyala soda mine pits in all the three soda pits are Microcystis, Oscillatoria, Anabaena, Cylindrospermopsis and Phormidium. For microcystin RR it was not found in any of the three soda pits. In the three Nyala soda pits the levels of microcystin LR ranging from 24.25 to 102.71 ppb were in excess of the WHO guidelines of 1 ppb for drinking water and 20 ppb for recreational swimming. For soda pit 2, the levels of microcystin YR and LY were 18.16 ppb and 28.93 ppb respectfully. Thus the presence of the microcystins in the soda pits poses a health hazard to the people who may use the water for a variety of uses.

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Reference

Carmichael W W (1989) Freshwater cyanobacteria (blue-green algae) toxins. In: C L Odell GV (Eds.),Natural Toxins: Characterization, Pharmacology and Therapeutics. Pergamon Press, Oxford pp. 3-6

Carmichael W W (1994) The toxins of cyanobacteria. Scientific American 270(1): 78-86

Codd G A, Bell S G, Brooks W P (1989) Cyanobacterial toxins in water. Water Science and Technology 21 (3): 1-13

Codd G A, Lindsay J, Young F M, Morrison L F, Metcalf J S (2005) Harmful cyanobacteria: from massmortalities to management measures, in J. Huisman, H.C.P. Matthijs and P.M. Visser (eds.), Harmful Cyanobacteria. Springer, Dordrecht, The Netherlands pp. 1-23

Falconer, I R, Beresford, A M, Runnegar, M T C (1983) Evidence of liver damage by toxin from a bloom of the blue green alga, Microcystis aeruginosa. Med. J 1: 511-514

Gumbo J R, Cloete T E (2011) The mechanism of microcystis aeruginosa death upon exposure to Bacillus mycoides. Journal of Physics and Chemistry of the Earth 36: 881-886

Hamed A F, Salem B B, & El-Fatah A H (2007) Floristic survey of Blue-Green Algae/Cyanobacteria in Saline-Alkaline Lakes of Wadi El-Natrun (Egypt) by Remote Sensing Application. Journal of Applied Sciences Research 3(6): 495-506

Jeleni M N, Gumbo J R, Muzerengi C, Dacosta F A (2012) An assessment of toxic metals in Soda Mine Tailingsand a Native Grass: A Case Study of an Abandoned Nyala Magnesite Mine, Limpopo, South Africa. 415-426. Water Pollution XI

Jonker C Z, van Ginkel C, Oliver T (2013) Association between physical and geo-chemical characteristics of thermal springs and algal diversity in Limpopo Province, South Africa. Water SA. 39: 95-103

Kamennaya NA, Ajo-Franklin C M, Northen T, & Jansson C (2012) Cyanobacteria as biocatalysts for carbonate mineralization. Minerals 2(4): 338-364

Keneck SL, Hrudey SE, Prepas EE, Motkosky N, Peterson H.G (1992) Odorous substances and cyanobacterial toxins in Prairie drinking water sources. Water Science and Technology 25 (2): 147-154

Lopez A. (1998) Phormidium http://www.cyanosite.bio.purdue.edu/images/lgimages/phorsp.jpg

Oberholster P J, Myburgh J G, Govender D, Bengis R, Botha A M. (2009) Identification of toxigenic microcystis strains after incidents of wild animal mortalities in the Kruger National park, South Africa. Ecotoxicol. Environ. Saf.

Panda H S, Nayak M, Das B, Parida B K, Jena J, Sukumar B, Panda S, Panda P K, Sukla L B. (2011) Survey and documentation of brackish water algal diversity from East Coast Region of Odisha, India.World Environment 1(1): 20-23

Pouria S. (1998) Fatal microcystin intoxication in hemodialysis unit in Caruaru, Brazil: Lancet 352**:** 16-21

Sellner K G, Doucette G J, Kirkpatrick G J. (2003) Harmful algal blooms: causes, impacts and detection. J Ind Microbiol Biotechnol 30: 383-406

Van Vuuren S J, Talyor J, Gerber A (2006) A guide for identification of algae in South Africa freshwaters. NorthWest University and Department of Water Affairs and Forestry.

World Health Organization (1998) Guidelines for drinking water quality, 2nd ed. Addendum to vol. 2. Healthcriteria and other supporting information. World Health Organization, Geneva: 95-110